A Chemical Study of the Grape Fruit

by C. W. Seibel

May 15th, 1913

Submitted to the School of Engineering of the University of Kansas in partial fulfillment of the requirements for the Degree of Bachelor of Science

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A CHEMICAL STUDY OF THE GRAPE FRUIT

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The Grape Fruit or Pomelo, as it is called, belongs to the family of Citrus Decumanas and is a native of the Malay Archipelago. It was first introduced into this country and the West Indies by Capt. Shaddock, early in the 18th century. For this reason it is sometimes called the Shaddock, although the true Shaddock is a pear-shaped fruit and not the round fruit cultivated and put on the market from Florida, the so-called Grape Fruit.[(1)] (2)*. The fruit probably gets its present name from the fact that it grows in bunches, sometimes as many as fifteen fruit in one cluster. This is quite remarkable when one stops to think that the fruits will average about 350 to 400 grams each. The tree from which we obtain the pomello attains a height of about thirty feet. The leaves are large and oval in shape, the flowers correspondingly large and pure white. The fruit itself has a smooth yellow skin very similar to that of a lemon, and is about five inches in diameter. It consists of first a thin, slick outer surface or skin which is generally a pale yellow, due no doubt to the oil found imprisoned in it.

(*) The numbers in parentheses refer to the corresponding numbers in the Bibliography.

Under this comes a rather thick layer of a white, sort of pithy material which will average about one-fourth inch in thickness. This white substance contains the bitter principle. Beneath this is the juicy part which is kept from the white by a thin skin or membrane.(3)

The juice is a murky, slightly yellow liquid which may be readily obtained by the use of a fruit press or by diging it out with a spoon. It contains some suspended matter which quickly cloggs a filter, making it difficult to separate. The best method found was to centrifuge the juice for fifteen minutes at a speed of 2500 r.p.m. This gets rid of most of the suspended substances, no more separating on standing.

If the juice is to be kept for some time, one-tenth gram of mercuric chloride per liter should be added, as the juice ferments quickly. Ten drops of a forty per cent solution of formaldehyde per liter were used and found insufficient to keep it. On fermenting, a light grey mould formed on the surface of the liquid, it then takes on a sour smell, but the acid content was found not to be greatly altered.

In the process of analysis the fruit was first measured to get the circumference and then weighed. The peel was then cut in segments and removed, and as much as possible of the adhering white substance, which clings to the inner skin of the fruit was also removed. This leaves the fruit in very good shape to place in a press for the expression of the juice. Before this is done, however, it is weighed and from this data the percentage of peel is found. Now the juice is pressed out and measured. The number of cubic centimeters times the specific gravity will give the weight of the juice from which the per cent may be found. If the seeds are now weighed, all the necessary data for the calculations of percentages of peel, juice, pumice and seeds are obtained.

The juice, as has been stated, contains much suspended matter, goes through four thicknesses of cheese cloth and so quickly clogs a common filter or even a Gooch that it is impossible to clarify it. In fact, it has been the experience of the author that the freshly extracted juice can not be entirely clarified. The best results were obtained by centrifuging at a speed of 2500 r.p.m. for fifteen minutes and siphoning off the top liquid. In this manner a comparatively clear juice can be obtained. This juice is easily attacked by a greyish mould, which forms in from two to three days time, depending on surrounding conditions.

The juice itself has a slightly acid taste due to the citric acid present. The amount of citric acid present in the juice may be found by titrating 6.8 c.c. of it with N/10 NaOH. Phenolphthalein makes a very good indicator for this purpose. The number of cubic centimeters of N/10 NaOH used divided by 10 gives directly the percentage of acid. (3).

The experiments carried on by the government have indicated a definite relation between the acidity of a ripe citrus fruit and the amount of sugar present. They also found that in an unripe fruit this relation did not hold. They determined that a ripe fruit would contain 5% of cane sugar to 1% acid. Thus an unripe fruit is easily told from a ripe one. (4).

Along this line is another thing which gives the government a good deal of trouble, that is frozen or frost bitten fruits. These bear no outward evidence of having been frozen; but the insides are pithy and dry. Such a fruit was obtained and found to contain only 35 c.c. of juice, whereas a ripe fruit of equal weight gave 226 c.c. Under U. S. Department of Agriculture Food Inspection Decision No. 150 Citrus Fruits are claimed adulterated if in any crate 15% or more show on a transverse section through the center, a marked drying of 20% or more of the exposed pulp. (4).

The juice of the Pomello contains on an average about ten per cent of solids, whereas according to Leach the orange contains 12.72% and government reports show the lemon to contain 11.23% of solids. (12).

The solid content is easily determined by putting into a tared aluminum dish 5 c.c. of the juice. This is then heated on a water bath for two hours, cooled in a desiccator and weighed. The difference in weight times 20 gives the percentage of solids per 100 c.c..

Work done by J. A. LeClerc and L. M. Tolman showed that the above process gave results which did not vary more than .15% from data collected by heating an equal sample in vaccum for one day at 70°C. (13).

The ash obtained by heating 25 c.c. of juice in a tared platinum dish in a muffle, till a white ash was obtained, runs considerably lower than either that of the orange or the lemon as determined by government chemists. (5).

The ash obtained in this manner may be divided into two parts: that soluble in water and that that is not. Both these ashes have a distinctly alkaline nature. The amount of alkalinity in each may be determined by titrating each separately with N/10 HCl, using phenolphthalein as an indicator. Before this is done, however, they must be separated. This is accomplished by adding 40 c.c. of hot water to the ash in the platinum dish and heating just below boiling for two hours. It is now filtered, washed and titrated. The material in the filter, the insoluble ash, is burned with the filter paper and dissolved in an excess of N/10

HCl -- about 10 cc. was found sufficient. This excess is then titrated with N/10 NaOH. The difference being the alkalinity of the insoluble ash over 25 c.c. of juice. (13)

The juice contains some phosphates and these are found in the ash and are therefore present in the two solutions which have just been titrated. They may be determined quantitatively in the following manner.

The neutral solutions of the soluble and of the insoluble ash are acidified with nitric acid and filtered if necessary, but in this case care must be taken to wash free from acids. The phosphate is precipitated from the two solutions, separately by adding while hot, an excess of ammonium molybdate. Digest this for 45 minutes on a water bath, filter and wash with a ten per cent solution of ammonium nitrate. Care must be taken that none of the precipitate goes through the filter, which it has a decided tendency to do. The best plan to prevent this is to use a good quality of quantitative filter paper and not let the liquid come over two-thirds of the way to the top. The yellow precipitate is then dissolved in N/2 NaOH and the excess NaOh titrated with N/10 HCl, phenolphthalein as indicator. The number of c.c. of NaOH N/2 used times 0.062 gives mil. gms. of P_2O_5 in the sample. (13).

In obtaining the refraction of the Grape Fruit juice, a Zeiss immersion refractometer was used, but owing to the murky condition of the material to be tested, no sharp line could be obtained. With the aid of the Abbé refractometer, however, some very good checks on readings were obtained. There was very little difference in the readings of juices from Florida, California or Cuban fruits. In fact the differences were no more on the different varieties than on different fruits from the same locality. (12).

No trouble was experienced in obtaining the specific gravity of the different juices. It was done on a very accurate Westphal balance, one that gave readings to the fourth decimal place. Specific gravities were taken at two different temperatures: at 15.6°C. and 20°C., the former being the larger, in nearly every case by eight in the last decimal place.

In determining the sugar content of the juice, both cane and invert sugar, a Soleil-Ventzke Saccharimeter of half-shade type was used. Then, again, the cloudiness of the juice caused some trouble. After a number of attempts, using various methods, it was found that the best results were obtained by adding 5 c.c. of lead sub-acetate, 5 c.c. of aluminum cream, which precipitates out organic matter, 25 c.c. juice and 25 c.c. water. This gives a solution which may be used in a

400 m.m. tube. (12). However, if the invert sugar reading is wanted for this solution the lead from the added lead sub-acetate must be precipitated and filtered out. To avoid that, it may be omitted and only 5 c.c. aluminum cream added to one volume of juice and 2 volumes of water. Now, however, it is best to use a 100 m.m. tube. (12).

In getting the reading for invert sugar, the clarified juice just used is mixed with 6 c.c. strong HCl and allowed to set, cold, over night. It is then placed in a 100 m.m. tube and the reading taken. (12).

The original intention was to obtain sufficient oil from the yellow outer peel of the fruit to determine such constants as Reichert Meisel No., Hainer No., specific gravity and refractive index. After much work on trying to get a pure oil, only negative results were met with. It might be well to state a few of the ways in which these results were obtained.

The first method tried was to get out the oil by distilling the peel with steam. Only odor was gotten however. Apparently no oil came over at all; and if so, it was so small a quantity as to be completely soluble in the water. An alcoholic extract was tried. In order to get this concentrated, extractions were made in a Soxhlet, renewing the peel when exhausted and using the same alcohol. This gave a concentrated alcoholic extract of the oil and the problem was then to get the oil out in a somewhat pure state. (12).

Both the plain method, using the Babcock milk flask, and Howard's modification of Michell's precipitation method for lemon oil were used. In both these cases, however, only about a drop of seemingly impure oil could be obtained from the extract, but judging from the color of the rest of the contents of the flask, not all of the oil was precipitated. Salting out the oil by adding NaCl and also H_2SO_4 and centrifuging was of no avail.

The next method tried was that of removing the oil by pressing. For this purpose a press made by Mr. Geo. O. Peterson for expressing the oil out of nuts was used. This press had worked so well for Mr. Peterson that it was thought feasible for use in removing the oil from the peel. The peel was first ground, after removing as much as possible of the white part, and packed in the press, tightly wrapped in four thicknesses of cheese cloth. The press was simply a hollow cylinder with holes drilled in the walls near the bottom and a snugly-fitting plunger which was forced down on the peel. The whole machine was placed in a hydraulic press. Trouble was experienced in getting the cheese cloth to hold; the peel would break it and force itself out through the holes. With four thicknessess of cheese cloth and four of heavy towling, a pressure of only some 18 tons could be applied without the peel breaking out. In this manner so little oil was obtained as to make it a complete failure. Finally, enough oil was obtained to get a reading on the Abbe' refractometer by bending the peel sharply and allowing the fine jets of oil which shot out to fall on the prisms of the instruments.

It might be well to give the method which is applied to lemons in extracting the oil. It, however, is a very slow and tedious process, being entirely too slow and costly for application here. The following article is taken from, "The Manufacturing of Flavoring Extracts" by E. M. Chace. "The work of expressing the oil is done entirely by men, women being rarely employed, as the work is quite laborious. The workers sit upon low stools, the skins being cleaned on the floor in front of them and a basket of the exhausted skins placed a little to one side. A small earthenware bowl 8 to 10 inches high and about the same diameter, is placed on the floor between the workman's knees. This bowl has at one side a lip, directly beneath which is a small concave depression, which serves to hold back the residue when the oil is poured into it. Across the top is placed a round stick of wood, an inch in diameter, so notched as to fit the widest part of the bowl. Across this stick is hung a flat sponge surmounted by another thicker one and finally a third which is cup-shaped, into which the lemon skin is inserted with the right hand, the left being used to press upon the sponge, the weight of the whole body being thrown into the motion. The lemon rind is then turned partly over and the pressure renewed. This is repeated three or four times, after which the skin is thrown into the waste basket. Each half rind is handled separately, receiving three or four pressings. From 1,600 to 2,200 of these half rinds produce only one pound of oil, the quantity depending on the size, ripeness and freshness of the fruit. It is said that green fruit produces rather more oil than ripe, and that lemons should be worked as soon as possible after picking. A good workman can produce from three to four pounds of oil a day for which he receives from forty to sixty cents."(10)

This method could doubtless be used here only, as one can see, it is far too expensive.

Professor E. H. S. Bailey noticed in a bulletin published at the University of Illinois by N. E. Goldthwaite the statement that the white part of oranges and lemons contained a good deal of pectin, which could be made into an extract and used in supplying the necessary pectin in fruit juices which contain little or none of it. He suggested that some interesting work on this line might be carried on with Grape fruit. (11)

This necessitated finding how to make jelly from fruit juices. It seems that in order to obtain a good jelly, which Mrs. Goldthwaite defines as a beautifully colored, transparent, palatable product obtained by so treating fruit juice that the resulting mass will quiver, not flow, when removed from its mould, a product with texture so tender that it cuts easily with a spoon, and yet so firm that the angles thus produced retain their shape; a clear product which is neither syrupy, gummy , sticky nor tough; neither is it brittle and yet it will break and does this with a distinct, beautiful cleavage which leaves sparkling characteristic faces, it is necessary that the juice be acid and that it contain a substance called pectin and added sugar. Whether the juice contains pectin or not, is easily determined by adding to some of the juice an equal part of alcohol. In case there is pectin present, a gelatine-like substance will be found in the hollow of the cup. The amount of this is some indication of the pectin present. (11).

Now a certain amount of pectin can only take care of a definite amount of sugar. If the sugar is in excess, a soft jelly is produced; and if the juice is deficient in sugar, a tough stringly jelly is most likely to be obtained. Since the pectin content varies greatly with every juice, no definite rule can be given as to the amount of sugar to be added. Mrs. Goldthwaite found however, that from 3/4 vol. sugar : 1 vol. juice to 1:1 was in most cases about right.

An extract of the white part of the rind of the Grape Fruit was made by cutting the white part free from the outer skin and chopping it up fine. This was soaked in enough water to cover it, over night; enough water added to make half water and half peel and boiled for about fifteen minutes. The juice was then strained through cheese cloth. This was found to contain an abundance of pectin.

Different percentages of this extract were tried with different percentages of sugar and the juice of the Grape Fruit, which in itself contains no pectin. It was found that half juice and half extract with three-fourths of the total volume of sugar added, gave the best results. The jelly had a very slightly bitter taste due to the glucoside in the peel, but this was hardly noticeable.

The bitter principle of the Grape Fruit is found in the white spongy material between the outer yellow skin and the juice itself. This bitter substance was found to be easily soluble in water and alcohol. Water extract of it was made by boiling some of it, obtained by cutting it off of the yellow outer peel, with water which had been slightly acidulated with H_2SO_4. This was intensely bitter when first made, but when tested two months later it was found to have lost all of its bitter taste and to be sweet. An alcoholic extraction, however, remained bitter. At first it was thought that the bitter taste must be due to an alkaloid and a comlete set of tests were run through for them. The method used was that outlined in Allen's Commercial Analysis.

There was a decided tendency to form an emulsion, but on standing, the liquid separated into well defined layers. The solvent with 30 c.c. of the bitter extract was sharply agitated in a separatory funnel for some time.

EXTRACTION OF ALKALOIDS BY IMMISCIBLE SOLVENTS

The liquid is warmed to about 45°, rendered distinctly acid with H_2SO_4 and agitated with an equal measure of petroleum ether.

Aqueous solution agitated with benzene and separated.

Aqueous liquid agitated with $CHCl_3$ and separated.

Aqueous liquid agitated with some petroleum ether to extract soluble $CHCl_3$, more petroleum ether added, NH_4OH in excess, agitated and separated.

Aqueous solution agitated with $CHCl_3$.

Aqueous liquid agitated with Amyl alcohol 60°C. & separated

Petroleum extracts from acid liquid	Benzene extracts from acid liquid	$CHCl_3$ extracts--	Petroleum ether extracts from ammonical solutions--	$CHCl_3$ extracts from NH_4OH solution.	Extracts:-	Aqueous solution contains--
<u>Bases</u>	<u>Bases</u>	<u>Bases</u>	<u>Bases</u>	<u>Bases</u>	<u>Bases</u>	
	Beberine (traces)	Cinchonine (doubtful)	Aconitine	Cinchonine	Narcine (slightly)	Berberine (traces)
	Caffeine	Narcotin (traces)	Atropine	Morphine (traces)	Morphine	Narcine
			Brucine			
			Coedine			
			Narcotine			
			Quinodine			
			Strychnine			
<u>Acids</u>	<u>Acids</u>	<u>Acids</u>				
Salicylic		Benzoic				

Future discoveries lead me to believe that the tendency to form an emulsion was due to the pectin; a gelatin-like substance which is present in large amounts in the white peel of the fruit. After running this clear down and finding no trace of any alkaloid, it was learned that it was probably a glucoside. If so, this at once accounted for the water extract turning sweet.

On going to the literature no method for the identification of a glucoside could be found. An Industrial fellow at Pittsburg was working on citrus fruits and through Mr. G. A. Bragg it was learned that the bitter principle was a glucoside. There is a variety of opinions as to its name and composition. It is generally stated to be naringin and also as isohesperidin.

A glucoside is a substance which is found in a number of plants along with an enzyme. In the fruit these two substances are separated by a thin skin wall. On rupture they come together and the glucoside is hydrolized by the enzyme; that is, it is broken up into glucose and water. The glucose is then fermented and the fruit spoils. The same results may be obtained by heating the glucoside in water; and better yet, if the water is slightly acidulated. In the alcohol, however, hydrolysis can not take place, and therefore the alcoholic solution before mentioned did not develop a sweet taste after standing, as did the water solution.

The Government has done very little work on the Grape Fruit up to the present time; and for this reason, there is little or no data to check against. However, the work done in this thesis tends to show some definite relations between the different constituents of the Grape Fruit.

For instance, as was stated by government officials, the ratio of the acid in a normal fruit to the sucrose present is a constant, there being present about 5% of sucrose for every 1% of acid.

Frozen fruit tend to become pithy and dry, only about one-sixth of the normal amount of juice being present.

The edible portion, that is, the juice, will average about 45% of the whole fruit.

The soluble and insoluble phosphates are present in equal amounts.

The oil contained in the skins of the fruit is a quick drying one, as far as could be determined.

The white part of the peel contains an intensely bitter substance, a glucoside called isohesperidin or naringin. It also contains a substance called pectin which may be gotten into the form of a water extract, and then may be used to supply fruit juices deficient in this substance, for the purpose of jelly-making.

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- D A T A -

BLUE SHIELD GRAPE FRUIT
Lakeland, Florida

10¢ each Bought of Wagstaff, Lawrence, Ks. Mch 13, 1913.

	I	II	III
Wt. Fruit	583.5 gms.	622.5 gms.	593.7 gms.
Peel	24.40%	23.8%	24.7%
Juice	41.20%	45.0%	44.0%
Seeds	2.52%	1.78%	1.30%
Pulp	31.88%	29.42%	30.00%
Diameter	34 cm.	35 cm.	34 cm.

Juice from above three = Juice No. I

CITRUS FRUIT EXCHANGE
Florida

3 for 25¢ Bought of F.H.Klock, Lawrence, Ks. Feb. 28, 1913.

	I	II	III
Wt. Fruit	467.15 gms.	450.37 gms.	503.84 gms.
Peel	19.77%	19.55%	23.53%
Juice	48.40%	42.50%	40.80%
Pulp & Seeds	31.83%		

Juice from above three = Juice No. II.

GRAPE FRUIT FROM
Island of Pines, Cuba

10¢ each Bought of F.H.Klock, Lawrence, Ks. Apr. 17, 1913

	I	II	III
Wt. Fruit	537.0 gms.	582.0 gms.	-----
Juice	33.4%	33.0%	-----
Peel	6.84%	11.3%	-----
Pumice	56.50%	50.34%	-----
Seeds	3.35%	3.96%	-----
Diamter	33 cm.	34 cm.	

GRAPE FRUIT FROM
California

5¢ Bought of F.H.Klock, Lawrence, Ks. Apr. 12, 1913

Wt. Fruit	341.00 gms.
Juice	26.20%
Peel	25.62%
Pumice	44.80%
Seeds	5.28%
Diameter	27.5

T O T A L   S O L I D S

JUICE NO. I.

Without centrifuging:

9.49 gms. solids per 100 c.c. juice.

With centrifuging: 1500 r.p.m.:

9.47 gms. solids per 100 c.c. juice.

JUICE NO. II

With centrifuge, 3000 r.p.m.:

9.95 gms. solids per 100 c.c. juice.

Without centrifuge:

11.08 gms. solids per 100 c.c. juice.

CUBAN JUICE

Centrifuged, 1500 r.p.m.:

10.49 gms. solids per 100 c.c. juice.

CALIFORNIA JUICE

Centifuged, 1500 r.p.m.:

11.2 gms. solids per 100 c.c. juice.

SPECIFIC GRAVITY

	20°C	15°C.
Juice No. I. centrifuged	1.0382	1.0390
Juice No. II. not centrifuged	1.0443	1.0458
Juice No. II. centrifuged	1.0405	1.0412
Cuban Juice	1.0419	1.0425
California Juice	1.0478	1.0487

REFRACTIVE INDEX AT 20° C.

Juice No. I.

Centrifuged 1.34605

Not centrifuged 1.34715

Juice No. II.

Centrifuged 1.3477

Non centrifuged 1.3489

Cuban Juice

1.3502

California

1.3481

Oil at 20°

1.4729

ACIDITY

Juice No. I	1.11% acid
Juice No. II	1.50% acid
Cuban Juice	0.817% acid
California Juice	1.17% acid

TOTAL ASH

Juice No. I. Centrifuged	0.4012 gms. per 100 cc
Juice No. II Centrifuged	0.2616 " " " "
Cuban Juice	0.3739 " " " "
California Juice	0.3389 " " " "

SOLUBLE AND INSOLUBLE ASH

	Sol.	Insol
Juice No. II	0.238%	0.013%
Cuban Juice	0.346%	0.123%
California	0.313%	0.011%

ALKALINITY OF SOLUBLE & INSOLUBLE ASH per 100 c.c.

	Sol.	Insol.
Juice No. II	22.48	9.16
Cuban Juice	40.80	10.32
California	36.52	8.40

P_2O_5

(In mil. gms. per 100 c.c. of juice)

	Sol.	Insol
Juice No. II	6.98	8.80
Cuban Juice	7.95	6.44
California Juice	9.60	7.08

SUCROSE

Juice No. I	4.5%
Juice No. II	7.65%
California juice	6.28%

GOVERNMENT REPORTS ON GRAPE FRUIT

(5)

Avg. Wt.	Solids	Insol. Solids	Ash	Sucrose
477.0	13.29	2.70	3.93	6.22
391.6	13.12	----	3.90	7.37
378.0	9.94	1.17	4.60	5.89

(8)

Average Weight, grams	357.00
Rind, per cent	23.50
Seeds, per cent	3.70
Juice, per cent	33.30
Solids in juice	13.20
Total sugars	9.50
Cane sugar	5.00
Acid in juice as citric	2.70